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(57) Abstract

A pharmaceutical composition for the oral administration of a therapeutically beneficial protein having a molecular weight of up to 100,000, comprising said protein, edetic acid and a carefully screened and tested dosage amount of protease inhibitor, said composition being provided with an enterocoating to assure passage through the stomach and release of said protein in the intestine.

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PHARMACEUTICAL COMPOSITIONS CONTAINING PROTEIN

The present invention relates to a pharmaceutical composition for the oral administration of a therapeutically-beneficial protein. More specifically, the present invention relates to a pharmaceutical composition containing insulin and constitutes a modification and improvement of the invention described in Israel specification 109,021.

In Israel specification 109,021 there is described and claimed a pharmaceutical composition for the oral administration of insulin, comprising insulin, edetic acid and a carefully screened and tested dosage amount of protease inhibitor, said composition being provided with an enterocoating to assure passage through the stomach and release of insulin in the intestine.

Insulin is a medicament particularly useful as a hypoglaemic agent being widely used by patients suffering from diabetes and is the only treatment for insulin-dependent diabetes mellitus.

In practice today insulin is administered only by injection. The everyday injection of insulin is very troublesome and causes considerable physical and even mental suffering for the patients. Several severe side effects such as lipodystrophy at the site of the injection, lipoatrophy, lipohypertrophy or occasional hypoglycemia have been noted and reported to occur.

To avoid the daily injection of the drug, the insulin pump has been developed in the last decade. This pump, however, also suffers from some of the disadvantages of the daily

injection. Since insulin is normally secreted into the portal vein, normally the liver is exposed to a greater insulin concentration than peripheral tissues. Insulin administered via the peripheral venous system to insulin-deficient diabetic patients results in a reduced concentration of insulin in the portal vein and the liver and hyperinsulinemia in the peripheral venous system. This may lead to an abnormal pattern of glucose disposal.

In order to overcome the difficulties caused by injection of insulin, rectal administration of insulin has recently been proposed, studied and developed.

Shichiri et al. (J.Pharm. Pharmac. 30,806-808,1978,), Bar-On et al. (Br.J. Pharmac. 73, 2-24,1981), and others tested the hypoglyceaemic effects of insulin mixed with polyoxyethylen lauryl ether or polyoxtethylene-20-cetyl ether by administering through the rectum. Ziv et al (Life Sciences, 29, 803-809,1981) tested the same effect with insulin mixed with bile salts. The insulin affected the blood glucose levels, by reduction of approximately 50%, with dose of 48 u/kg.

In a further article by Ziv, Kidron, Bar-On and Berry (Life Sciences, 31, pp. 2837-2841, 1982) insulin was used as a model for proteins in general to discover the theoretical question of protein absorption through the intestine and it was found that in the presence of the strong detergent effect of deoxycholic acid and soybean trypsin inhibitor, biologically active macromolecules such as insulin could be effectively absorbed from the intestine.

Similarly, in a British Pat. No. 1,563,311 there is described and claimed a pharmaceutical composition for

rectal administration which comprises insulin, a carrier suiting the composition for rectal administration, and an agent for increasing the rate of absorption of the insulin into the body on rectal administration of the composition, the agent comprising at least one material selected from (a) nonionic polyoxyethylene ether surface active agents having an HLB value of 6 to 19 and wherein the average number of polyoxyethylene units is 4 - 30, (b) anionic surface active agents, (c) cationic surface active agents. (d) ampholytic surface active agents, (e) bile acids and (f) alkali metal salts of bile acids and amounting to 0.001 to 0.5 times the weight of the carrier. In U.S. Pat. Nos. 4,434,159 and 4,164,573 there are described similar insulin containing pharmaceutical compositions for rectal administration.

Thus the administration of insulin through the portal system of the human rectum in suppository form or further along the intestinal tract, e.g., by enema-like introduction is suggested and taught by said articles and patent.

Neverthless it has been found that only part of the insulin is absorbed through the portal system from the human rectum and rectal administration also represents a major inconvenience for the patient.

Various attempts have been made in the past to administer insulin orally. In one study it was shown that administration of liposome-entrapped insulin caused a significant reduction of blood glucose levels in diabetic rats. (Dapergolas, G and Gregoriadis, Lancet ii,824 827,1976) Patel and Ryman (FEBS Letters, 62, 60-63, 1976) showed that insulin administered orally entrapped in liposomes is effective in diabetic rats. Papahadjopoulos

and Sjoka (U.S. Pat. No 4,235,871) suggested to use liposomes to encapsulate insulin and Sears (U.S. Pat. No. 4,145,410) used synthetic phosphatidyl compounds to stabilize the liposomes against lipolysis.

Another approach for insulin enhanced activity is the addition of an adjuvant such as choline to the insulin injections (U.S. Pat. No. 2,563,070).

In U.S. Patent 4,579,730 there is described and claimed a pharmaceutical composition for the oral administration of insulin comprising insulin, a bile acid or alkali metal salt therof, said bile acid being selected from the group consisting of cholic acid, chenodeoxycholic acid, taurocholic acid, taurochenodeoxycholic acid, glycocholic acid, glycochenocholic acid, 3ß-monohydroxychloric acid, lithocholic acid, 3a-hydroxy-12-ketocholic acid, 3α-hydroxy-12-ketocholic acid, 12α, 3ß-dihydrocholic acid, and ursodesoxycholic acid, and a protease inhibitor, said composition being provided with an enterocoating to assure passage through the stomach and release in the intestine.

After further research and development it has now been surprisingly found that contrary to the teachings of said patent it is not necessary to include bile salts in an oral composition in order to enhance the absorption of insulin or other therapeutically beneficial proteins from the intestinal luman to the blood circulation and that edetic acid, otherwise known as (ethylenedinitrilo) tetraacetic acid (EDTA), is more effective than bile salts for this purpose.

Thus, according to the present invention, there is now provided a pharmaceutical composition for the oral

administration of a therapeutically beneficial protein having a molecular weight of up to 100,000, comprising said protein, edetic acid and a carefully screened and tested dosage amount of protease inhibitor, said composition being provided with an enterocoating to assure passage through the stomach and release of said protein in the intestine.

It will be realized that none of the said publications teaches or suggests the novel pharmaceutical composition of the present invention which includes the use of protease inhibitors to protect the desired protein against proteolysis and the use of enterocoating of the active mixture.

Among the proteins contemplated for use in the compositions of the present invention are insulin, having a molecular weight of 6,000, glucagon, having a molecular weight of 3,550, interferon, having a molecular weight of 28,000, growth hormone, having a molecular weight of between 21,500 and 47,000, human serum albumin, having a molecular weight of about 69,000, erythropoietin, having a molecular weight of about 34,000 and granulacyte colony stimulating factor (GCSF), having a molecular weight of from about 30,000 to about 34,000.

It is to be noted that human serum albumin is not considered to be a pharmaceutically-active component; however, it can be used in the context of the present invention as a therapeutically-beneficial carrier for an active component.

Human insulin including human insulin genetically reproduced or any insulin such as, for example, the insulin obtained from cows (bovine), pigs or whales, can be used as the insulin for compositions if this invention. Furthermore, metal complexes of insulin such as the zinc complex of

insulin as well as protamine zinc insulin and globin zinc insulin may be also used as the insulin compositions of this invention.

The protease inhibitor used in the compositions of the present invention can be any material which has the ability to inhibit any proteolytic activity.

Practical examples of such protease inhibitors include aprotinin (Trasilol^{R} of Bayer), Pentamidine isethionate, antipain, tosylamide-phenylethyl-chloromethyl ketone (TPCK), phenylmethyl sulfonyfluoride (PMSF), pepstatin, trypsin inhibitor, Acetone, Alcohols, guanidium, a2-macroglubulin, TLCK, Chelating agents of Zn, Iodoacetate, a1-antitrypsin, Zn, Antithrombin III, leupeptin, Trypsin inhibitor from soy bean, trypsin inhibitor from hen egg white, trypsin inhibitor from chicken egg white, etc.

Some of the above protease inhibitors might be toxic in large doses and therefore, if chosen, the use and dosage therof must be carefully screened and tested. Furthermore it has now been found that there is a need for a specific protease inhibitor for each species. For example: the best protease inhibitor for the rat is trasylol (aprotinin), for the dog it is SBTI. (Kidron, M., Krausz, M.M., Raz, I.,Bar-On, H. and Ziv, E. The absorption of insulin from the intestine in dogs. Tenside, Surf. Det., 26, 352-354,1989.) Therefore, there is still a need to determine the best protease inhibitor for humans although it is believed that SBTI, trasylol or a combination thereof will produce the desired effect.

The amount of insulin in a composition is 20-50 u/kg in rats and expected to be about 0.5-3 u/kg in humans. Preferred

dosages for humans are about 1-2 u/kg/treatment with three treatments a day, however sustained release microencapsulation could allow treatment to be reduced to once or twice a day.

EDTA as a chelating agent, is used for the treatment of metal poisoning. Since EDTA is not absorbed from the gastrointestinal tract, oral administration is contra-indicated as therapy in case of metal poisoning, but in case of oral administration of insulin, according to the present invention, there is an advantage in giving a drug that is not absorbed from the gastrointestinal tract.

When administered as a chelating agent, the recommended daily dosage for adults (injection) is 50mg/kg of body weight to a maximum dose of 3 g in 24 hours. For children the recommended daily dosage (injection) is 40 mg/kg of body weight.

When using EDTA as an insulin enhancer, according to the present invention, the recommended dosage per capsule (oral administration) will be 2-5 mg/kg body weight, which is well below the above amounts, which have already been approved by the authorities for administration to the human body.

The enterocoating and possible microencapsulation of the mixture provides protection for the insulin against decomposition in the stomach and for the slow release of the mixture consituents in the intestinal tract.

The enterocoating is carried out by methods known per se in the art, e.g. according to Remington Pharmaceutical Sciences, p. 1614-1615 (1975,15th Edition. Mack Pub. Co.) and Theory and Practice of Industrial Pharmacy, Lackman,

Liberman & Canig, p. 116-117, 371-374 (1976, 2nd Ed) as is the enteric microencapsulation (Theory and Practice of Industrial Pharmacy ibid. pp 420-438).

Eudragit (an acrylic polymer) is one of the most common materials for enterecoated tablets or capsules. The use of acrylic polymers for the coating of pharmaceutical preparations is a well known technique which is successfully employed for the production of pharmaceuticals. On the basis of toxicological studies, a daily intake of 2 mg Eudragit/kg or 150 mg for an average adult person, may basically be regarded as safe in humans. Higher doses may be used and have been accepted by different health authorities, depending on the relevant toxicological data and on the intended application. Particularly important for the critical evaluation of the toxicology is the fact that the Eudragit Acrylic Polymers are neither absorbed nor metabolised by the body, but rather are completely eliminated without being transformed.

While the invention will now be described in connection with certain preferred embodiments in the following examples so that aspects thereof may be more fully understood and appreciated, it is not intended to limit the invention to these particular embodiments. On the contrary, it is intended to cover all alternatives, modifications and equivalents as may be included within the scope of the invention as defined by the appended claims. Thus, the following examples which include preferred embodiments will serve to illustrate the practice of this invention, it being understood that the particulars shown are by way of example and for purposes of illustrative discussion of preferred embodiments of the present invention only and are presented in the cause of providing what is believed to be the most

useful and readily understood description of formulation procedures as well as of the principles and conceptual aspects of the invention.

Example 1:

A solution was prepared for direct intestinal administration to the dog, in final volume of 2ml.

Formulation with 100 mg bile salts.
Insulin: 2U/kg, SBTI:2mg/kg

Formulation with 50mg EDTA
Insulin: 2 U/kg, SBTI: 2mg/kg

Time (min)	Glucose (mg%)	Insulin (uu/ml)	Glucose (mg%)	Insulin (uu/ml)
0	78	8	89	4
10	77	10	80	60
20	73	2	53	33
30	73	3	50	28
40	76	8	72	4
50	73	2	81	4

Formulation with 50mg EDTA,
Insulin: 1 U/kg, SBTI: 2mg/kg

Time (min)	Glucose (mg%)	Insulin (uu/ml)
0	76	7
10	76	32
20	58	17
30	53	5
40	63	3
50	68	3
60	72	1

Example 2:

A solution was prepared for direct intestinal administration to the dog, in final volume of 2 ml.

Formulation with 100 mg bile salts. Insulin: 8U/kg, SBTI:2mg/kg			Formulation with 50mg EDTA Insulin: 3U/kg, SBTI:3mg/kg.	
Time (min)	Glucose (mg%)	Insulin (uu/ml)	Glucose (mg%)	Insulin (uu/ml)
0	75	13	81	1
10	71	121	76	181
20	47	53	39	113
30	48	26	27	49
40	64	19	41	16
50	67	15	37	3
60	-	-	50	8
70	-	-	69	3
80	-	-	74	1

Discussion:

It has now been surprisingly found, as illustrated in the above examples that using EDTA (Disodium Tetraacetate) as an enhancer, is much more effective than bile salts. We have done more than 120 experiments on normal dogs. We have found that direct administration (of our formulation) into the intestine of the dog (using a cannula), resulted in the raising of blood insulin levels, and lowering of blood glucose levels of up to 50% and more from base line. When we compare the use of bile salts and the use of EDTA in direct administration into the intestine of the dog it is apparent that EDTA is more effective.

Example 3:

A solution was prepared for direct intestinal administratioj to the dog, in final volume of 2 ml.

Formulation with 50 mg EDTA mg Glucagon, 809 mg SBTI			Formulation with 75 mg EDTA 2 3 mg Glucagon, 80 mg SBTI in 3		
Time (min)	Glucose (mg%)	Glucagon (pg/ml)	Time (min)	Glucose (mg%)	Glucagon (pg/ml)
0	97	86	0	76	166
5	104	947			
12	132	144	7	82	1508
18	123	160	12	93	1632
25	108	305	17	80	317
30	128	178	22	69	705
40	115	216	32	97	253
50	123	157	42	95	280
60	96	122			

2mg glucagon-direct administration to the intestine control experiment			1mg glucagon-i.m. injection control experiment		
Time (min)	Glucose (mg%)	Glucagon (pg/ml)	Time (min)	Glucose (mg%)	Glucagon (pg/ml)
0	87	238	0	77	148
7	85	213	8	128	1627
12	85	332	13	127	1668
17	86	247	18	115	1365
29	81	216	28	89	995
34	79	142	38	84	714
			48	73	866

It will be evident to those skilled in the art that the invention is not limited to the details of the foregoing illustrative examples and that the present invention may be embodied in other specific forms without departing from the essential attributes thereof, and it is therefore desired that the present embodiments and examples be considered in all respects as illustrative and not restrictive, reference being made to the appended claims, rather than to the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced therein.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition for the oral administration of a therapeutically beneficial protein having a molecular weight of up to 100,000, comprising said protein, edetic acid and a carefully screened and tested dosage amount of protease inhibitor, said composition being provided with an enterocoating to assure passage through the stomach and release of said protein in the intestine.

2. A pharmaceutical composition according to claim 1, wherein said protein is selected from the group consisting of insulin, having a molecular weight of 6,000, glucagon, having a molecular weight of 3,550, interferon, having a molecular weight of 28,000, growth hormone, having a molecular weight of between 21,500 and 47,000, human serum albumin, having a molecular weight of about 69,000, erythropoietin, having a molecular weight of about 34,000 and granulacyte colony stimulating factor (GCSF), having a molecular weight of from about 30,000 to about 34,000.

3. A pharmaceutical composition for the oral administration of insulin, comprising insulin, edetic acid and a carefully screened and tested dosage amount of the protease inhibitor, said composition being provided with an enterocoating to assure passage through the stomach and release if insulin in the intestine.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL96/00055

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/18, 38/26, 38/27, 38/28, 38/55
US CL :514/2, 4; 530/303, 308, 350, 362
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2, 4; 530/303, 308, 350, 362

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN, APS
search terms: chelating agent, peptide, insulin, entercoating

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,579,730 A (KIDRON ET AL.) 01 April 1986, see entire document.	1-3
Y	US 4,476,116 A (ANIK) 09 October 1984, see entire document, expecially column 10-12.	1-3
Y	US 5,167,947 A (GEARY) 01 December 1992, see entire document, especially column 4.	1-3
Y	Lee, V.H.L. Protease Inhibitors and Penetration Enhancers As Approaches to Modify Peptide Absorption. Journal of Controlled Release. 1990. Vol. 13. pages 213-223.	1-3

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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